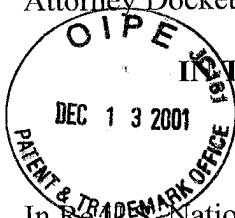


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Attorney Docket No. 3551 P 003



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
U.S. NATIONAL FILING UNDER 35 USC §371**

In Re U.S. National Patent Application of:  
Conor MULROONEY et al.

U.S. Serial No. 09/936,382  
Filed September 10, 2001  
From: PCT/GB00/00921 filed March 13, 2000

# For: ENZYMATICAL CATALYSED SIGNAL AMPLIFICATION

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**SECOND  
PRELIMINARY AMENDMENT**

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COMMISSIONER FOR PATENTS  
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Prior to examining the application identified above, please amend the application as follows.

Note that this amendment is being submitted in accordance with 37 CFR 1.121.

IN THE CLAIMS:

Please add the following new claims:

36. A method for detecting a target molecule according to claim 3, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 11.
  
  37. A method for detecting a target molecule according to claim 5, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 11.

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38. A method for detecting a target molecule according to claim 1, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 12.

, cont  
B

39. A method for detecting a target molecule according to claim 3, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 12.

40. A method for detecting a target molecule according to claim 5, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 12.

41. A method for detecting a target molecule according to claim 12, prior to said detection step additionally comprising performing step (ii) of a method according to claim 1.

42. A method for detecting a target molecule according to claim 11, prior to said detection step additionally comprising performing step (ii) of a method according to claim 3.

43. A method for detecting a target molecule according to claim 12, prior to said detection step additionally comprising performing step (ii) of a method according to claim 3.

44. A method for detecting a target molecule according to claim 11, prior to said detection step additionally comprising performing step (ii) of a method according to claim 5.

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45. A method for detecting a target molecule according to claim 12, prior to said detection step additionally comprising performing step (ii) of a method according to claim 5.

*B cont*

46. A method for detecting a target molecule according to claim 12, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 1.

47. A method for detecting a target molecule according to claim 11, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 3.

48. A method for detecting a target molecule according to claim 12, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 3.

49. A method for detecting a target molecule according to claim 11, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 5.

50. A method for detecting a target molecule according to claim 12, said amplification moiety of said locator probe or additional locator probe from said final amplification step

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comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 5.

51. A method for detecting a target molecule according to claim 3, the step of detecting any bound amplification template comprising the steps of:

- B-Conf*
- i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
  - ii) detecting any bound detection probe.

52. A method for detecting a target molecule according to claim 5, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

53. A method for detecting a target molecule according to claim 18, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

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54. A method for detecting a target molecule according to claim 19, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

55. A method for detecting a target molecule according to claim 12, the step of detecting any bound amplification template comprising the steps of:

- i) treating said sample, locator probe and amplification template with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and
- ii) detecting any bound detection probe.

56. A method according to claim 3, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

57. A method according to claim 5, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

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58. A method according to claim 11, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

*B1 cont*

59. A method according to claim 12, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

60. A method according to claim 3, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

61. A method according to claim 5, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

62. A method according to claim 11, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

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63. A method according to claim 12, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

*B1a*  
64. A method according to claim 3, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

65. A method according to claim 5, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

66. A method according to claim 11, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

67. A method according to claim 12, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

68. A method according to claim 3, comprising two repeats.

69. A method according to claim 5, comprising two repeats.

70. A method according to claim 11, comprising two repeats.

71. A method according to claim 12, comprising two repeats.

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72. A method according to claim 3, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

*(31 cont)* 73. A method according to claim 72, the unreacted reagents being selected from the group of locator probe, amplification template, and detection probe.

74. A method according to claim 5, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

75. A method according to claim 74, the unreacted reagents being selected from the group of locator probe, amplification template, and detection probe.

76. A method according to claim 11, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

77. A method according to claim 76, the unreacted reagents being selected from the group of locator probe, amplification template, and detection probe.

78. A method according to claim 12, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

79. A method according to claim 78, the unreacted reagents being selected from the group of locator probe, amplification template, and detection probe.

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No new matter is being added through these amendments. Applicant respectfully requested entry of the above amendments.

Respectfully submitted,

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Susan Franklin